Supplementary Information

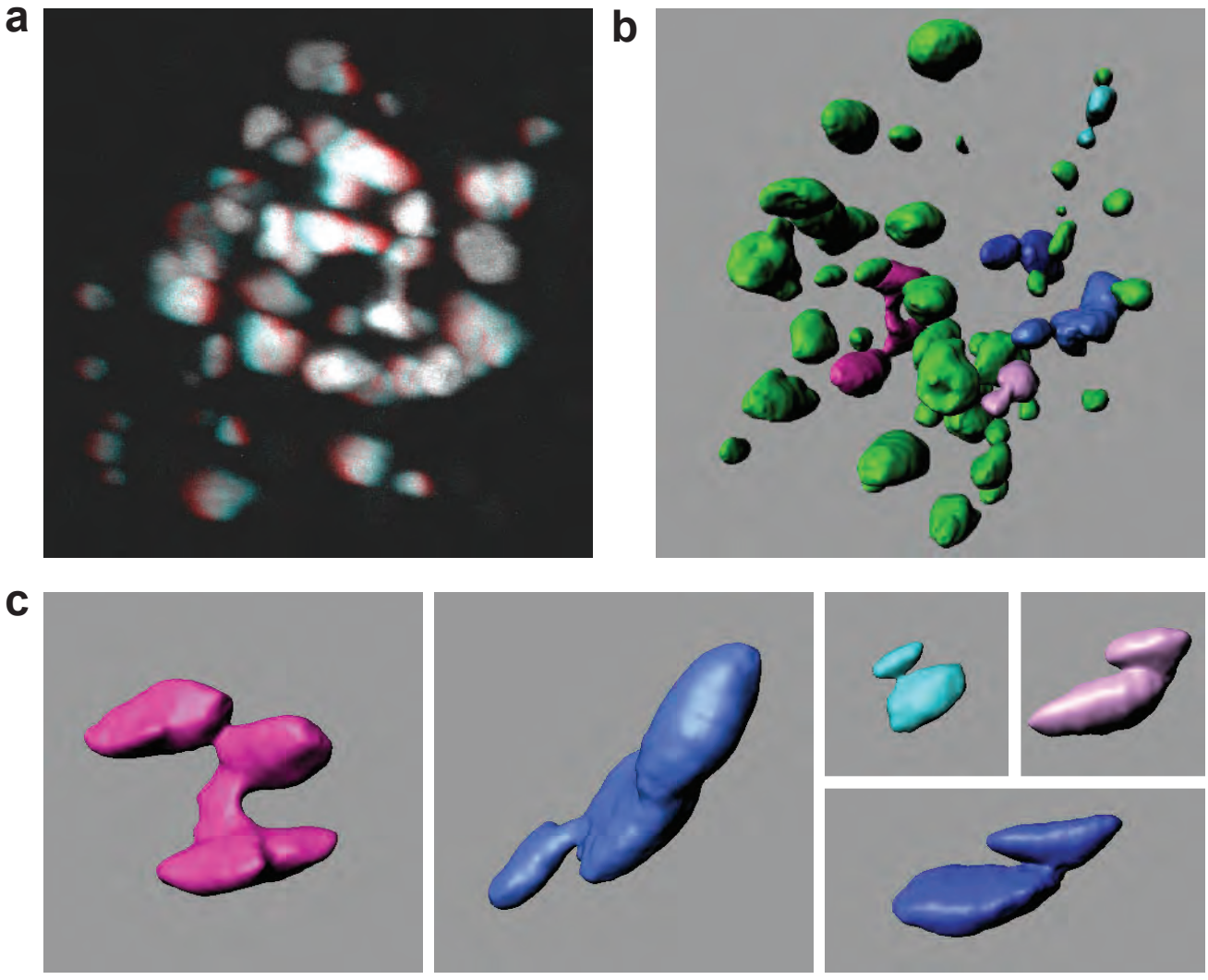
Supplemental Figure legends

Suppl. Fig. 1. Visualization of highly condensed bivalents from *A. tesselata* GVs in late prophase of meiosis I. DAPI-stained samples were imaged by two-photon microscopy using a Carl Zeiss long working-distance C-Apochromat 40x N.A. 1.1 objective. **(a)** Stereo 3D projection of the chromosomes from a late prophase GV (265 µm diameter). Scale bar corresponds to 5 µm. **(b)** Iso-surface rendering of the chromosomes shown in (a) using the software package IMARIS 6.3 (Bitplane). In this sample 35 bivalents were readily identifiable as isolated objects. **(c)** One object corresponding to three bivalents in close proximity and four objects corresponding to two bivalents each brought the total number of bivalents to 46, consistent with the number expected if premeiotic endoreplication had occurred. A minimum of 40 clearly discernable bivalents were counted in two additional *A. tesselata* GVs of similar size. Physical proximity between some bivalents prohibited definitive identification of all 46 bivalents in these samples. **(d)** Animation of the rendered chromosomes shown in (b).

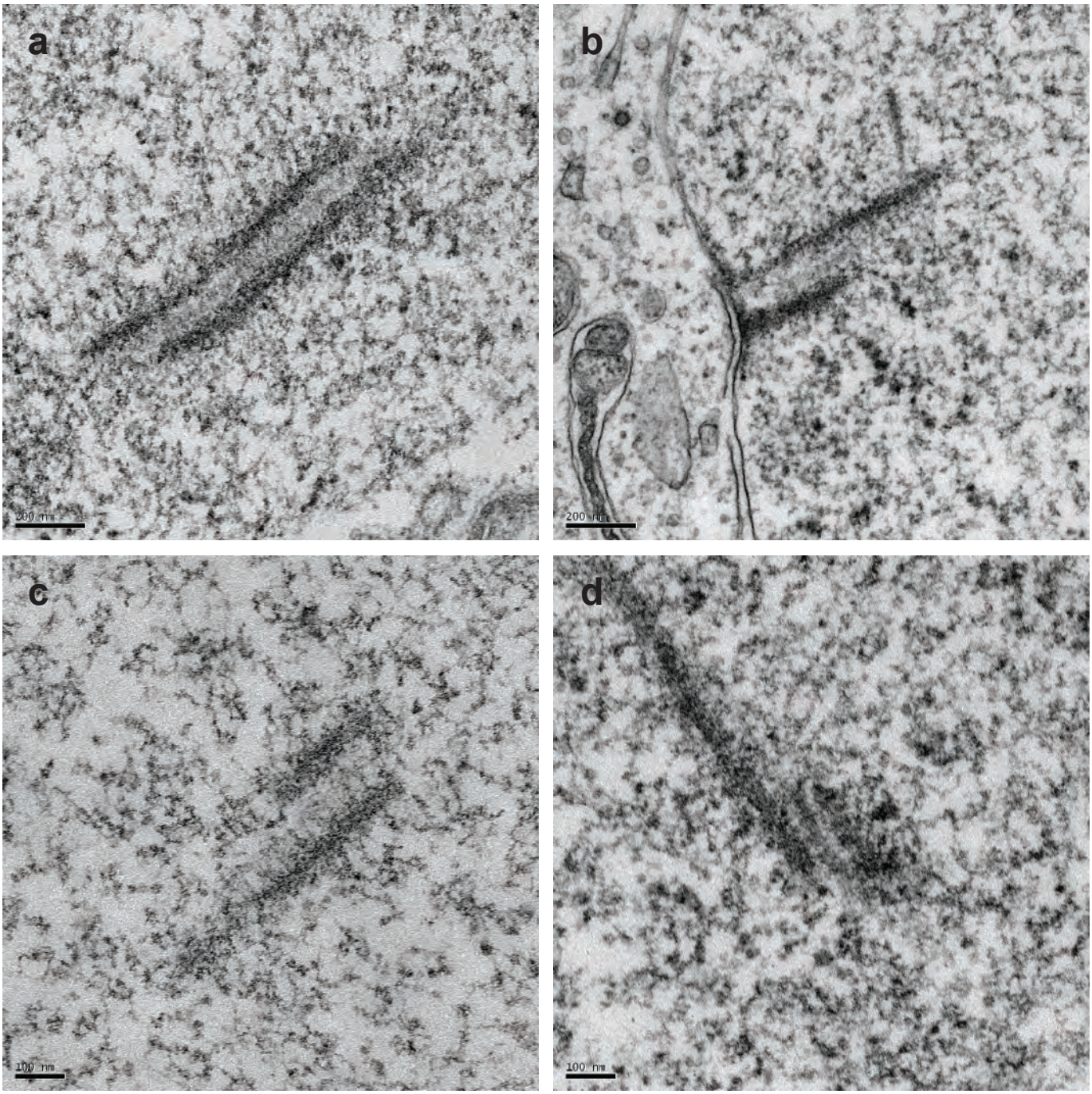
Suppl. Fig. 2. Synaptonemal complexes visualized by electron microscopic examination of thin sections from *A. neomexicana* oocytes. Scale bars correspond to 200 nm in **(a)** and **(b)** and to 100 nm in **(c)** and **(d)**.

Suppl. Fig. 3. Assessment of pairing partners using a fluorescent $(CCAAGG)_2CC$ hybridization probe on an *A. neomexicana* GV. **(a)** A $(CCAAGG)_2CC$ locked nucleic

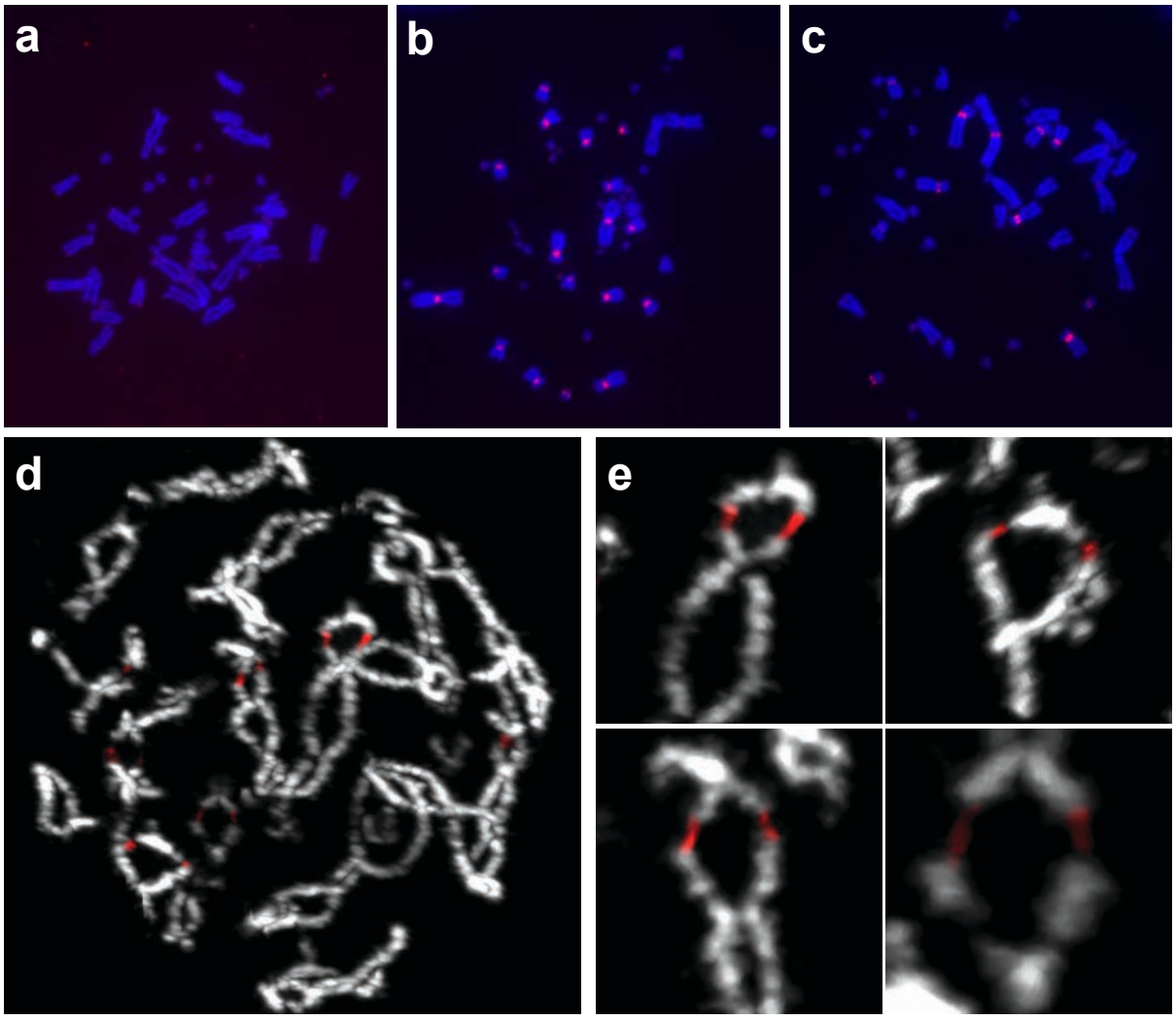
acid probe (red) does not hybridize strongly to *A. inornata* chromosomes. **(b)** 18 chromosomes from *A. tigris* are labeled by the same probe. **(c)** Chromosomes inherited from *A. tigris* but not from *A. inornata* are identified by the fluorescent probe in *A. neomexicana.* The inheritance of one set of chromosomes from *A. tigris* and the other from *A. inornata* leads to the expectation of nine foci in *A. neomexicana* cells. In reality ten brightly stained loci were observed. The tenth signal could be the result of homolog pairing and cross-over resulting in homozygozity at that locus. Alternatively, a different chromosome may have acquired the repeat region either before or after the hybridization event that gave rise to *A. neomexicana*. **(d)** Projection of a subset of images from an *A. neomexicana* GV visualized by confocal microscopy. DAPI-stained chromosomes are shown in white and the CCAAGG probe in red. **(e)** Close-up of four representative areas visualizing paired fluorescence signals.



Lutes, et al.; Suppl. Figure 1



Lutes, et al.; Suppl. Figure 2



Lutes, et al.; Suppl. Fig. 3

Table 1. DNA Quantification

GV	Diameter (μm)	Chromosome Volume (μm^3)	Chromosome Volume Ratio
gularis #1	71	1304	1.00
gularis #2	63	1023	0.79
gularis #3	71	995	0.76
gularis #4	77	1535	1.18
gularis #5	91	1296	0.99
gularis #6	57	878	0.67
gularis #7	65	1430	1.10
tesselata #1	77	2901	2.23
tesselata #2	81	2585	1.98
tesselata #3	99	3069	2.35
tesselata #4	62	3216	2.47
tesselata #5	99	2844	2.18

Measurements of GV sizes and volume occupied by chromosomes in each sample.